

EFFECT OF FERMENTED *Sauropus androgynus* LEAVES ON BLOOD LIPID FRACTION AND HAEMATOLOGICAL PROFILE IN BROILER CHICKENS

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ABSTRAK

Penelitian ini dilakukan untuk mengevaluasi pengaruh daun katuk (*Sauropus androgynus*) fermentasi terhadap konsentrasi fraksi lipid darah dan profil hematologis pada ayam broiler. Seratus dua belas ekor ayam broiler dikelompokkan ke dalam 7 kelompok perlakuan. Satu kelompok diberi pakan tanpa daun katuk fermentasi sebagai kontrol, dan enam kelompok lainnya diberi pakan yang mengandung daun katuk yang difermentasi oleh *Neurospora crassa*, *Lactobacillus* sp. (EM4) atau *Saccharomyces cerevisiae* pada tingkat 25 g/kg atau 50 g/kg pakan. Hasil penelitian menunjukkan bahwa perlakuan berpengaruh tidak nyata terhadap kolesterol, high-density lipoprotein cholesterol (HDL-c), low-lipoprotein cholesterol (LDL-c) dan indeks aterogenik, very low-density lipoprotein cholesterol (VLDL-c) dan konsentrasi trigliserida ($P>0,05$). Hasil penelitian ini menunjukkan bahwa daun katuk fermentasi secara nyata mempengaruhi red blood count (RBC), white blood count (WBC), packed cell volume (PCV), trombosit dan erythrocyte sedimentation rate (ESR) ($P<0,05$), tetapi tidak berpengaruh pada hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), dan mean corpuscular volume (MCV). Disimpulkan bahwa daun katuk yang difermentasi oleh *Neurospora crassa* meningkatkan jumlah trombosit. Di samping itu, daun katuk fermentasi tidak dapat menurunkan konsentrasi trigliserida dan kolesterol darah serta indeks aterogenik pada ayam broiler. Pemberian 50 g daun katuk yang difermentasi oleh EM4 bersifat racun bagi ayam broiler.

Kata kunci: daun katuk fermentasi, fraksi lipid, profil hematologis, ayam broiler

ABSTRACT

This study was conducted to evaluate effect of fermented *Sauropus androgynus* leaves on blood lipid fractions and haematological profiles in broilers. One hundred and twelve broilers were distributed to 7 treatment groups. One group was fed diets without *Sauropus androgynus* leaves as the control, and other six groups were fed *Sauropus androgynus* leaves fermented by *Neurospora crassa*, *Lactobacillus* sp. or *Saccharomyces cerevisiae* at level of 25 g or 50 g/kg diet. Experimental results showed that the treatments had no effect on cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and atherogenic index, very low-density lipoprotein cholesterol (VLDL-c) and triglyceride concentration ($P>0.05$). It was shown that fermented *Sauropus androgynus* leaves significantly affected red blood count (RBC), white blood count (WBC), packed cell volume (PCV), trombosit dan erythrocyte sedimentation rate (ESR) ($P<0.05$), but it had no effect on haemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), dan mean corpuscular volume (MCV). It was concluded that *Sauropus androgynus* leaves fermented by *Neurospora crassa* increased trombocyte number. Fermented *Sauropus androgynus* leaves had no beneficial effect on lowering blood triglyceride and cholesterol concentrations, and atherogenic index in

broiler chickens. Feeding 50 g EM4 fermented *Sauropus androgynus* leaves per kg diet might be toxic for broiler chickens.

Keywords: *fermented Sauropus androgynus, lipid fractions, haematological profiles, broilers*

INTRODUCTION

Rapid increases in the body weight was accompanied by a higher abdominal fat and carcass fat in broiler chickens. This excess fat accumulation was a waste of energy for poultry producers and consumers (Musa *et al.*, 2006). Therefore, the selection of lean breed of broiler chicks become an important value in recent years. However, measuring abdominal fat and carcass fat content for selecting lean broiler chicks required slaughtering broiler and fat analysis which require many facilities and high cost. Therefore, it is required a simple method to predict those values. Experimental results showed that lipid concentration in serum was a good parameter to predict lipid content in animals. Musa *et al.* (2007) reported that abdominal fat was positively correlated with triglycerides, very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL), and negatively correlated with cholesterol and high-density lipoprotein (HDL) in chickens.

On the other hand, experimental results showed that there is correlation between concentration of blood fat such as cholesterol with the risk of atherosclerosis, heart coronary, stroke and other metabolic diseases (Willett, 2012). This phenomenon could be minimized by feed additive supplementaion. *Sauropus androgynus* leaves were rich in linolenic acid (31.75%), palmitic acid (12.14%), chlorophyll (9.33%), benzoic acid (8.58%) and alkaloid (7.2%) (Samad *et al.*, 2014) which might be benefit for reducing lipid fractions concentration. Previous study showed that *Sauropus androgynus* leaves reduced serum cholesterol (Santoso *et al.*, 2010). In additon, it has been shown that *Sauropus androgynus* leaves and its extract reduced fat contents of meat and egg (Santoso *et al.*, 2005; Subekti, 2007). However, the reduction of fat contents in broiler chicks was still under 25% which was still had not economic significant in industries (Santoso *et al.*, 2005). Furthermore, *Sauropus androgynus* leaves tended to lower body weight gain (Santoso and Sartini, 2001). This will decrease the profit of broiler industries because in Indonesia the price of broiler meat is stiiil based on body weight rather than carcass quality.

Sauropus androgynus leaves contained antinutritions such as oxalate, saponin, tannin, oligosacharide and inhibit calsium absorption (Santoso, 2014), and caused lung abnormality (Hasyimoto *et al.*, 2013).

To improve the value of *Sauropus androgynus* leaves, they could be fermented. Fermentation had an important role in improvement of nutritional and functional properties of foods. Cleavage of food proteins by microbial or indigenous proteases yields the bioactive peptides, leading to substantial increases in the biological properties of the food (Steinkraus, 2002). Moreover, fermented food products are a good source of peptides and amino acids (Rajapakse *et al.*, 2005). Fermentation increased nutritional values and feed utuilization in poultry, and protein and soluble protein, and converted protein to peptide and amino acid (Susi, 2012), increased nutrient digestibility (Chiang *et al.*, 2010), increased the activity of trypsin, lipase, and protease (Feng *et al.*, 2007), improved amino acid balance (Ari *et al.*, 2012; Susi 2012), reduced crude fiber and reduced antinutritions such as trypsin inhibitor (Ari *et al.*, 2012), tannin (Olaniyi dan Mehdizadeh, 2013) and phytic acid (Ari *et al.*, 2012; Olagunju dan Ifesan, 2013), phenol, phytin phosphorus and oxalate (Olagunju dan Ifesan, 2013), saponin (Olaniyi dan Mehdizadeh, 2013), and alkaloid (Shu *et al.*, 2010). Fermentation by *Lactobacillus* or *Saccharomyces cerevisiae* (Dordevic *et al.*, 2010) and *Bacillus subtilis* (Juan dan Chou, 2010) increased antioxidant properties. Experimental results also showed that fermentation increased mineral availability. Furthemore, there is no study of fermented *Sauropus androgynus* leaves influence on haematological profiles in broiler chickens.

The present study was conducted to evaluate effect of fermented *Sauropus androgynus* leaves on blood lipid fractions and haematological profiles in broiler chickens. It was hypothesized that feeding fermented *Sauropus androgynus* leaves reduced blood cholesterol, triglyceride and LDL but increased HDL concentration, whereas haematological profiles were also improved.

MATERIALS AND METHODS

Animals

One hundred and twelve broiler chicks were obtained from commercial hatchery, and all feedstuffs were purchased from commercial plants. Chemicals used for analysis were of analytical grade. The experimental diets contained protein 19% and Metabolizable Energy (ME) 3,150 kcal/kg. The composition of experimental diet was published elsewhere (Santoso *et al.*, 2015). From 1 to 14 days of age, supplemental heat was provided. Broiler chickens were maintained on the floor in a house under continuous lighting.

Fermented *Sauropus androgynus* Treatments

At 15 days of age, one hundred and twelve broiler chicks were selected and distributed to 7 treatment groups with 4 replicates of 4 broiler chicks each as follows: 1) Broiler were fed diets without *Sauropus androgynus* leaves as the control (P0); 2) Broilers were fed diets contained 25 g *Sauropus androgynus* leaves fermented by *Neurospora crassa*/kg diet (P1); 3) Broilers were fed diets contained 50 g *Sauropus androgynus* leaves fermented by *Neurospora crassa*/kg diet (P2); 4) Broilers were fed diets contained 25 g *Sauropus androgynus* leaves fermented by *Lactobacillus sp* (EM4)/kg diet (P3); 5) Broilers were fed diets with 50 g *Sauropus androgynus* leaves fermented by *Lactobacillus sp* (EM4)/kg diet (P4); 6) Broilers were fed diets contained 25 g *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*/kg diet (P5); 7) Broilers were fed diets contained 50 g *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*/kg diet (P6).

Feed and water were provided *ad libitum*. Broiler chickens were weighed individually on a weekly basis, and feed consumption was recorded daily.

Sampling and Data Analysis

At the end of experiment (35 days of age), 4 broiler chickens in each treatment group were selected and blood samples were collected by puncturing wing vein using 3 ml syringe and collected to bottles without anticoagulant agent. Immediately after blood collection, the sample bottles were gently shaken to prevent lysing of the blood (Ekeh *et al.*, 2010).

The packed cell volume (PCV) was

determined by the micro haematocrit method (Coles, 1986). The haemoglobin concentration was determined using the cyanmethemoglobin method while the erythrocyte (red blood count, RBC) was carried out using the haemocytometer method (Schalm *et al.*, 1975). The thrombocyte count was determined using the Resch-Ecker method (Brown, 1976) and the total leukocyte (white blood count, WBC) was determined using haemocytometer method (Schalm *et al.*, 1975) while the differential leukocyte count was determined using the Leishman technique (Coles, 1986).

To obtain the plasma, blood samples were collected to bottle with anticoagulant agent, and centrifuged at 3000 rpm. Blood serum obtained were then analyzed for triglyceride, cholesterol, high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c).

Total cholesterol and LDL-c were determined by the method of Kulkarni (2006) and triglyceride concentrations were determined by the method of Fossati and Prencipe (1982). Very low-density lipoprotein cholesterol (VLDL-c) was calculated by the following equation:

$$\text{VLDL-c} = \text{cholesterol} - (\text{HDL-c} + \text{LDL-c})$$

In order to predict the risk of atherosclerosis occurrences, atherogenic index was calculated by the equation:

$$\text{Atherogenic index} = [(\text{Total cholesterol} - \text{HDL-c}) / (\text{HDL-c})]$$

Internal organs (liver, heart, pancreas, intestine, gizzard, spleen) were removed and weighed, and then calculated by the equation:

$$\text{Internal organ (\%)} = \frac{\text{internal organ weight}}{\text{live weight}} \times 100\%$$

Toxicity score was calculated by the equation:

$$\text{Toxicity (\%)} = \frac{\text{liver} + \text{spleen weights}}{\text{live weight}} \times 100\%$$

Statistical Analysis

All data were subjected to analysis of variance (Toutenburg and Shalabh, 2009) and if it was significantly different it was further tested by Duncan's multiple range test.

RESULTS AND DISCUSSION

Lipid Fraction Concentrations

Experimental results showed that the treatments had no effect on blood cholesterol, HDL-c, LDL-c, atherogenic index, VLDL-c and triglyceride concentration ($P > 0.05$) as shown in

Table 1. Lipid Fraction Concentration in Plasma of Broiler Chickens

	P0	P1	P2	P3	P4	P5	P6	SD
Cholesterol	113.8	118.5	116.5	114.5	123.3	114.0	126.3	15.1 ^{ns}
Triglyceride	18.0 ^a	26.0	39.8	26.8	21.8	23.8	28.3	15.7 ^{ns}
HDL-c	70.5	68.8	70.3	66.0	69.5	67.3	74.3	9.0 ^{ns}
LDL-c	39.3	42.8	38.0	42.8	48.8	40.3	45.8	9.0 ^{ns}
VLDL-c	4.0	6.9	8.2	5.7	5.0	6.4	6.2	3.5 ^{ns}
Atherogenic index	0.61	0.72	0.66	0.73	0.77	0.69	0.70	0.11 ^{ns}

P0= control; P1= diets with 2.5% *Sauropus androgynus* leaves fermented by *Neurospora crassa*; P2= diets with 5% *Sauropus androgynus* leaves fermented by *Neurospora crassa*; P3= diets with 2.5% *Sauropus androgynus* leaves fermented by *Lactobacillus sp* (EM4); P4= diets with 5% *Sauropus androgynus* leaves fermented by *Lactobacillus sp* (EM4); P5= diets with 2.5% *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*; P6= diets with 5% *Sauropus androgynus* leaves fermented by *Saccharomyces cerevisiae*.

Table 1. This finding did not agree with the observation of Kamalia *et al.* (2014) who found that supplementation of *Sauropus androgynus* leaves at level 1-3% in broiler chicken diets increased HDL-c and tended to decrease LDL-c. It was unknown why fermentation did not change blood lipid fractions of broiler chickens. Although fermented *Sauropus androgynus* did not reduce LDL-c and cholesterol it might still be benefit for broiler chickens, because *Sauropus androgynus* leaves had a high level of antioxidant and anti-inflammatory activities (Madhu *et al.*, 2014). This antioxidant property of these leaves would inhibit LDL-c oxidation and therefore it might inhibit atherosclerotic lesion development (Koyama *et al.*, 2006).

The present study was in contrary with the observation Kamalia *et al.* (2014), Zain (2011) and Santoso *et al.* (2005) who reported that supplementation of unfermented *Sauropus androgynus* leaves extract reduced total cholesterol, triglyceride, and LDL-c but increased HDL-c concentrations in serum of broiler chickens. Fermentation might reduce antinutritious such as tannin and saponin (Olaniyi dan Mehdizadeh, 2013), and alkaloid (Shu *et al.*, 2010). It was known that these compounds had antilipid properties (Afrose *et al.*, 2010; Santoso *et al.*, 2010). Therefore, no beneficial effect of fermented *Sauropus androgynus* leaves might partly be caused by the reduction of these antilipid compounds.

Various studies showed that lipid fraction

concentration of broilers varied. Triglyceride, cholesterol, HDL-c, LDL-c and VLDL-c ranged from 19.09 to < 150 mg/dL, 52-157.8 mg/dL, >22-118.15 mg/dL, 35.56-<130 mg/dL, and 3.82-24.06, respectively (Basmacioglu and Ergul, 2005; Musa *et al.*, 2007; Prasad *et al.*, 2009; Daneshyar *et al.*, 2011). It appeared that age and strain of broiler chickens affected lipid fraction concentrations. This study, therefore, showed that lipid fraction concentrations in the plasma of broiler chickens were still in normal range.

Haematological Profile

Table 2 presents haematological profile of broiler chickens. Results showed that fermented *Sauropus androgynus* leaves significantly affected red blood count (RBC), white blood count (WBC), PCV, erythrocyte sedimentation rate (ESR) and thrombocyte ($P<0.05$), but it had no effect on haemoglobin (Hb), MCH, MCV and MCHC ($P>0.05$). WBC was higher in P4 as compared to other treatment groups. Although it was not significantly different, Hb in P2, P3, P5 and P6 tended to be increased as compared to P0. PCV of P3 was higher than P0, P1, P2 and P4, whereas RBC of P3 was higher than P1 ($P<0.05$) but not significant with other treatment groups. ESR of P1 was the highest, whereas thrombocyte was higher in P1 and P2 as compared with P0, P4 and P6 ($P<0.05$).

Higher thrombocyte in *Neurospora crassa* fermented *Sauropus androgynus* leaves – but still in normal range – indicated that immunity system

Table 2. Haematological Profile of Broiler Chickens

	P0	P1	P2	P3	P4	P5	P6	SD
RBC (x 10 ⁶ /mm ³)	2.63 ^{ab}	2.40 ^b	2.61 ^{ab}	2.85 ^a	2.56 ^{ab}	2.74 ^{ab}	2.72 ^{ab}	0.16 [*]
WBC (x 10 ³ /mm ³)	26.55 ^b	25.63 ^b	26.54 ^b	27.12 ^b	36.06 ^a	27.00 ^b	27.10 ^b	1.0 [*]
Lymphocyte, %	96.0	97.0	96.0	96.0	93.0	96.0	96.0	2.8 ^{ns}
Thrombocyte, x 10 ³ /mm ³)	2.25 ^b	5.25 ^a	6.25 ^a	3.75 ^{ab}	2.25 ^b	3.50 ^{ab}	2.0 ^b	1.6 [*]
PCV, %	33.75 ^b	32.25 ^b	33.00 ^b	37.75 ^a	32.75 ^b	35.25 ^{ab}	34.75 ^{ab}	1.5 [*]
Haemoglobin, g/ml	10.4	9.88	10.48	11.95	9.95	11.13	10.83	1.1 ^{ns}
MCH, pg	39.25	40.75	39.75	41.75	38.5	40.0	39.5	2.0 ^{ns}
MCV, fl	129.00	135.50	128.75	133.75	129.25	129.25	128.75	4.8 ^{ns}
MCHC, %	30.0	30.0	30.5	31.0	29.75	30.75	30.25	0.9 ^{ns}
ESR, mm/hour	3.25 ^c	8.25 ^a	3.25 ^c	4.0 ^c	5.0 ^b	5.0 ^b	4.5 ^{ab}	1.5 [*]

Note: see Table 1.

Table 3. Internal Organ Weights in Broiler Chickens

	P0	P1	P2	P3	P4	P5	P6	SD
Liver	2.14	2.08	2.20	2.28	2.85	2.13	2.18	0.45 ^{ns}
Heart	0.40	0.35	0.41	0.39	0.46	0.37	0.36	0.08 ^{ns}
Spleen	0.14	0.17	0.15	0.15	0.19	0.10	0.12	0.06 ^{ns}
Intestine	3.27	2.86	3.18	3.28	3.86	3.13	3.12	0.49 ^{ns}
Gizzard	1.83	1.93	2.06	2.03	2.21	1.84	1.78	0.24 ^{ns}
Pankreas	0.29	0.30	0.33	0.26	0.45	0.28	0.30	0.08 ^{ns}
Toxicity	2.27 ^b	2.25 ^b	2.36 ^b	2.43 ^b	3.04 ^a	2.23 ^b	2.30 ^b	0.47 [*]
Mortality	0.00	0.00	0.00	0.00	1.00	0.00	0.00	

Note: see Table 1.

was improved. Thrombocytes were found to phagocytose bacteria. Furthermore, oxidative burst activity was generated upon challenge of thrombocytes with various *Salmonella* strains, *E. coli*, three other bacterial species, and zymosan A (Wigley *et al.*, 1999). Therefore, thrombocytes might play an important role in innate immunity to bacteria in the chicken (Farzana, 2014; Wigley *et al.*, 1999).

Suprayogi *et al.* (2007) found that fermented

Sauropus androgynus leaves extract tended to increase RBC, PCV, Hb and WBC. Furthermore, they stated that papaverine and polyunsaturated fatty acids contained in *Sauropus androgynus* leaves may be as precursors in the eicosanoids biosynthesis such as prostaglandin, prostacycline, thromboxane, lipoxines dan leukotrienes. Adesua and Onibi (2014) reported that inclusion of fermented wheat bran to broiler diets did not change PCV, Hb and RBC, whereas Adeyemi *et*

al. (2008) showed no change in PCV, RBC and Hb was observed with a decreased in WBC. A higher WBC in P4 might relate to toxicity indication. This prediction was supported by higher toxicity score, mortality and the tendency of higher liver and spleen weight.

An increase in ESR of P1, P4 and P5 might be the result of increase tendency in serum LDL concentration (Khan and Zafar, 2005), as this lipoprotein got coated on the surface of RBC therefore increasing its ESR.

The average number of RBC in chicken range from $2.0 \times 10^6/\text{mm}^3$ to $3.5 \times 10^6/\text{mm}^3$ (Onibi *et al.*, 2011; Swenson, 1984; Smith and Mangkoewidjojo, 1988). Normal value of hemoglobin in chicken range from 6.5-13.0 g/mL (Onibi *et al.*, 2011; Orawan and Aengwanich, 2007; Swenson, 1984), PCV range from 22-43% (Onibi *et al.*, 2011; Orawan and Aengwanich, 2007; Smith and Mangkoewidjojo, 1988), WBC range from $11.4-30 \times 10^3/\text{mm}^3$ (Swenson, 1984; Orawan and Aengwanich, 2007); lymphocyte range from 24-84% (Smith and Mangkoewidjojo, 1988; Orawan and Aengwanich, 2007); thrombocyte range from $3-33 \times 10^9/\text{l}$ (Smith and Mangkoewidjojo, 1988), erythrocyte sedimentation rate (ESR) range from 3.0-12 mm per hour, MCV 132.21 fl (Orawan and Aengwanich, 2007), MCH 33.0-47.0 pg (Orawan and Aengwanich, 2007) and MCHC 26.35 (Orawan and Aengwanich, 2007). Based on the above data it was concluded that the haematological profiles of broiler chickens in this study were still in normal range.

Internal Organ Weights and Toxicity Score

Experimental results showed that fermented *Sauropus androgynus* leaves had no effect on liver, heart, spleen, intestine, gizzard, and pancreas ($P > 0.05$), whereas toxicity was significantly different ($P < 0.05$) as shown in Table 3. Toxicity was higher in P4 than P0, P1, P2, P3, P5 and P6. Although it was not significantly different P4 had the highest internal organ weight including liver and spleen. It appear that feeding 50 g EM4 fermented *Sauropus androgynus* leaves/kg diet had toxic properties as indicated by higher toxicity score and mortality in this group. This prediction was supported by the higher WBC in this treatment group. Adeyemi *et al.* (2008) reported that inclusion of fermented cassava root to broiler diet did not change relative liver, spleen and gizzard weights but it increased heart and

pankreas weights.

CONCLUSION

Fermented *Sauropus androgynus* leaves had no beneficial effect on lowering cholesterol and triglyceride concentrations, and atherogenic index. Although fermented *Sauropus androgynus* did not reduce plasma LDL-c and cholesterol concentrations it might still be beneficial for broiler chickens, because a high level of antioxidant and anti-inflammatory activities in *Sauropus androgynus* leaves might inhibit LDL-c oxidation and therefore it might inhibit atherosclerotic lesion development. *Neurospora crassa* fermented *Sauropus androgynus* leaves increased the number of thrombocyte. Broiler chicken fed 25 g EM4 fermented *Sauropus androgynus* leaves/kg diet had higher PCV and RBC, whereas broiler chicken fed 50 g EM4 fermented *Sauropus androgynus* leaves/kg diet had higher WBC. Feeding 50 g EM4 fermented *Sauropus androgynus* leaves/kg diet might be toxic for broiler chickens.

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